Short Communication

Impact of Phlebotomine Sand Flies on U.S. Military Operations at Tallil Air Base, Iraq: 3. Evaluation of Surveillance Devices for the Collection of Adult Sand Flies

DOUGLAS A. BURKETT,[1] RONALD KNIGHT,[2] JAMES A. DENNETT,[3] VAN SHERWOOD,[4] EDGAR ROWTON,[5] and RUSSELL E. COLEMAN[5,6]

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ABSTRACT We evaluated the effectiveness of commercially available light traps and sticky traps baited with chemical light sticks for the collection of phlebotomine sand flies. Evaluations were conducted at Tallil Air Base, Iraq, in 2003. In an initial study, a Centers for Disease Control and Prevention (CDC)-style trap with UV bulb collected significantly more sand flies than did an up-draft CDC trap, a standard down-draft CDC trap (STD-CDC), or a sticky strap with a green chemical light stick. In a subsequent study, we found that the addition of chemical light sticks to sticky traps resulted in a significant increase in the number of sand flies collected compared with sticky traps without the light sticks. These data indicate that 1) the CDC light trap with an UV bulb is an effective alternative to the standard CDC light trap for collecting phlebotomine sand flies in Iraq, and 2) that the addition of a chemical light stick to a sticky trap can result in a field-expedient tool for the collection of sand flies.

At the start of operation Iraqi Freedom in March 2003, U.S. forces rapidly established operations at Tallil Air Base (TAB), located ≈10 km west of An Nasiriyah in southern Iraq. As part of a surveillance program designed to assess and mitigate the risk of leishmaniasis, Centers for Disease Control and Prevention (CDC)-style light traps were used from April 2003 until October 2004 to monitor sand fly abundance at TAB (Coleman et al. 2006).

Although both light traps and sticky traps have been commonly used for the collection of sand flies (Lane et al. 1988, Mutero et al. 1991, Alexander 2000, Orndorff et al. 2002), few studies have systematically evaluated the efficacy of different types of light traps or the use of light in combination with a sticky trap. Alexander (2000) reported that a potential disadvantage of light traps was that they might preferentially sample females of certain species that are highly phototropic and suggested that light traps had limited value in ecological studies of sand flies. However, Fryauff and Modi (1991) and Davies et al. (1995) determined that light trap collections were comparable to biting collections for *Phlebotomus papatasi* Scopoli in Egypt and sand flies in the Peruvian Andes, respectively, whereas Rioux et al. (1982) reported that adhesive traps provided results similar to human bait.

Because we had already established an ongoing sand fly surveillance program using unbaited CDC light traps, we decided to compare the efficacy of this trap with two commercially available light traps (a CDC trap that used an UV light source and an updraft CDC light trap) and a sticky trap that is routinely available during military deployments. We also decided to determine whether the addition of a light source to a sticky trap would increase the number of sand flies collected. Because chemical light sticks of a variety of different colors are readily available during military deployments, we chose to evaluate six of the most commonly found colors.

Materials and Methods

Light Trap Evaluation. Light trap evaluations were conducted from 15 to 25 June 2003. Each trapping period ran from 2100 to 0700 hours (local time) the next day. The four trap types evaluated included 1) a standard CDC-style downdraft light trap (model 1012, John W. Hock, Gainesville, FL), 2) a CDC-style light trap using an UV bulb (model 1312, John W. Hock), 3) an updraft CDC-style light trap (Trapkit1 with updraft lid adapter, American Biophysics Corp., East Greenwich, RI), and 4) a green chemical light stick (Cyalume Ominglow Corp., West Springfield, MD)

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[1] 407th Air Expeditionary Group, United States Air Force, Tallil Air Base, Iraq.
[2] CSSB 16, United States Navy, Iraq.
[3] 792nd Medical Detachment, United States Army, Iraq.
[4] 787th Medical Detachment, United States Army, Iraq.
[5] Walter Reed Army Institute of Research, Silver Spring, MD 20910.
[6] Corresponding author: 520th Theater Army Medical Laboratory, United States Army, Tallil Air Base, Iraq (e-mail: russell.coleman@us.army.mil).

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Fig. 1. Placement of a sticky trap baited with a chemical light stick in a typical sand fly habitat at Tallil Air Base, Iraq.

attached to a 20- by 7-cm "cockroach" sticky trap (Fig. 1). Carbon dioxide or other supplemental attractants were not used. Evaluations were conducted in a 2,500-m^2 area of desert scrub habitat intermixed with piles of brick and stone building rubble, in an area with no prior vector control or insecticidal activities. Traps were placed ≈0.5 m above the ground and 30 m or more apart. After each trap night, the number of sand flies collected in each trap was determined. Sand flies were placed in vials with 75% ethanol and shipped to the Walter Reed Army Institute of Research (WRAIR) for identification to species. The trap evaluation consisted of two replicates of a 4 by 4 Latin square design. Trap, day, and location effects were evaluated using a three-way analysis of variance (ANOVA) (SAS Institute 1995). Trap data were transformed to $\log_{10}(x + 1)$ before analysis. Multiple comparisons were made using Duncan multiple range test ($\alpha = 0.05$).

Chemical Light Color Evaluation. Trials were conducted nightly from 2000 to 0600 hours the next day during the period 18–26 July 2003. Evaluations were conducted in assorted desert scrub and rubble habitats associated with rodent burrows. Six-inch blue, green, yellow, orange, red, and infrared (IR) chemical light sticks that were rated for 12 h were used. The sticky trap/light sticks were placed on the ground with the sticky surface and light stick facing up (Fig. 1). Traps were at least 30 m apart. After each trap night, sand flies stuck on each trap were counted. Because of the difficulty of removing the sand flies from the sticky board, the specimens were not sexed or saved for further identification. Controls included a plain sticky trap with no chemical light and a 20 by 10 cm index card coated with a thin layer of Castor oil. The trap evaluation consisted of an 8 by 8 Latin square design where trap, day, and location effects were evaluated using a three-way ANOVA (SAS Institute 1995). Trap data were transformed and analyzed as described for the light trap evaluation. Multiple comparisons were made using Duncan multiple range test ($\alpha = 0.05$).

Table 1. Collection of sand flies by using various commercially available CDC-style light traps and a sticky-trap baited with a chemical light stick

Species	Mean (SEM) no. of sand flies collected in each trap[a]				P value
	Down-Draft UV	Up-Draft CDC	Down-Draft CDC	Chemlight	
Female	61.5 (15.3) a	7.3 (2.6) b	3.3 (1.3) b	0.3 (0.2) c	0.0001
Male	41.0 (11.0) a	4.4 (1.5) b	2.5 (0.6) b	0.3 (02) c	0.0001
Total	102.9 (26.9) a	11.6 (3.6) b	5.8 (1.9) b	0.5 (0.3) c	0.0001

[a] Arithmetic means within each row having the same letter are not significantly different ($n = 8$ nights; $\alpha = 0.05$).

Results

Light Trap Evaluation. In total, 966 sand flies (578 females and 388 males) was collected during the eight trap nights. The UV CDC-style (UV-CDC) trap collected significantly more female sand flies (61.5 ± 15.3, mean ± SEM) than all other traps combined ($F = 49.8$, $P < 0.0001$) (Table 1). While the updraft CDC-style (UD-CDC) trap was the second most effective trap for collecting female sand flies (7.3 ± 2.6). The standard CDC-style (STD-CDC) trap (3.3 ± 1.3) and sticky trap (0.3 ± 0.2) baited with a chemical light (ST-CHEM) captured the fewest female sand flies. There were no significant location ($F = 2.8$, $P = 0.071$) or day ($F = 0.91$, $P = 0.52$) effects. In total, 184 sand flies collected in the light trap study were identified at least to genus, to include 41 *P. papatasi* (22.3%), 30 *Phlebotomus alexandri* Sinton (16.3%), two *Phlebotomus sergenti* (Parrot) (1.1%), and 111 *Sergentomyia* spp. (60.3%) (Table 2). Because only a few randomly selected sand fly specimens were identified from each trap, no statistical analyses were conducted on the individual sand fly species captured with the specific traps. However, the UV-CDC and UD-CDC traps seemed to be the most effective for collecting *P. alexandri* and *P. papatasi*, respectively (Table 2). The UD-CDC trap caught a smaller portion of *Sergentomyia* spp. than did either the UV-CDC or the STD-CDC traps.

Colored Chemical Light Sticks. In total, 434 sand flies was collected on the sticky traps during the 8-d collection period. Males and females were not differentiated or identified. Arithmetic means, standard errors, and significant differences for combined male and female sand flies are shown in Fig. 2. All colors of chemical light sticks in the visible spectrum captured significantly more sand flies than did the infrared-baited sticky traps, the sticky traps without light and than did the index cards coated with castor oil ($F = 3.36$, $P < 0.006$). No color of trap captured significantly greater numbers of sand flies than did any other color in the visible light range. There were significant day ($F = 3.17$, $P < 0.009$) and location ($F = 8.46$, $P < 0.006$) effects.

Table 2. Species of phlebotomine sand flies collected in various types of trap

Trap	No. sand flies collected (% of trap total)				Total
	P. papatasi	*P. alexandri*	*P. sergenti*	*Sergentomyia* spp.	
UV-CDC	13 (13.4)	24 (24.7)	0 (0.0)	60 (61.9)	97
STD-CDC	5 (13.2)	4 (10.5)	1 (2.6)	28 (73.7)	38
UD-CDC	23 (46.9)	2 (4.1)	1 (2.0)	23 (46.9)	49
Total	41 (22.2)	30 (16.3)	2 (1.1)	111 (60.3)	184

Discussion

As of November 2004, >1,100 cases of cutaneous leishmaniasis had been confirmed in U.S. military personnel deployed to Iraq and Afghanistan (Lay 2004). Because of the ongoing threat of leishmaniasis to military, humanitarian, and other operations being conducted in the Middle East, it is critical that preventive medicine and vector control personnel maximize their ability to monitor sand fly populations, evaluate control efforts, identify disease/vector foci, and suppress vector populations to mitigate the threat of leishmaniasis to deployed personnel. The identification of the most effective methods of collecting phlebotomine sand flies is key to this effort.

In this study, the overall number of medically important phlebotomine sand flies and the species composition of flies collected differed significantly among trap designs, ranging from a nightly mean of 102.9 ± 26.9 for the UV-CDC trap to 0.5 ± 0.3 for the chemical light-stick baited trap. The UV-CDC trap was clearly the most effective trap for collecting all species of phlebotomines, especially *P. alexandri*, the primary suspected vector of visceral leishmaniasis in Iraq. However, large numbers of nontarget insects greatly extended the processing time of nightly collections. Surprisingly, the literature does not reference studies that have used UV light traps for the collection of sand flies in the Middle East or North Africa.

Because of the difficulty of accurate identification, not all papers evaluating the use of surveillance techniques have assessed differences in the collection of individual species of sand flies. The overall species composition collected during our investigation agrees with what others have found in central Iraq, with the most abundant species including three known vectors (*P. papatasi*, *P. alexandri*, and *P. sergenti*) of parasites causing human leishmaniasis (Al-Azawi and Abul-Hab 1977, Abul-Hab and Al-Hashimi 1988). The various light traps assessed in our study were excellent tools for the collection of *P. papatasi.* Said et al. (1986) and Beavers et al. (2004) also used light traps to collect large numbers of *P. papatasi* in Egypt. However, Lewis (1971) found that *P. papatasi* was negatively phototrophic in Yemen, and Lane et al. (1988) reported that CDC-style light traps captured significantly fewer sand flies (including *P. papatasi*) in Jordon than did sticky traps with and without chemical light sticks. Lane et al. (1988) found no significant difference in

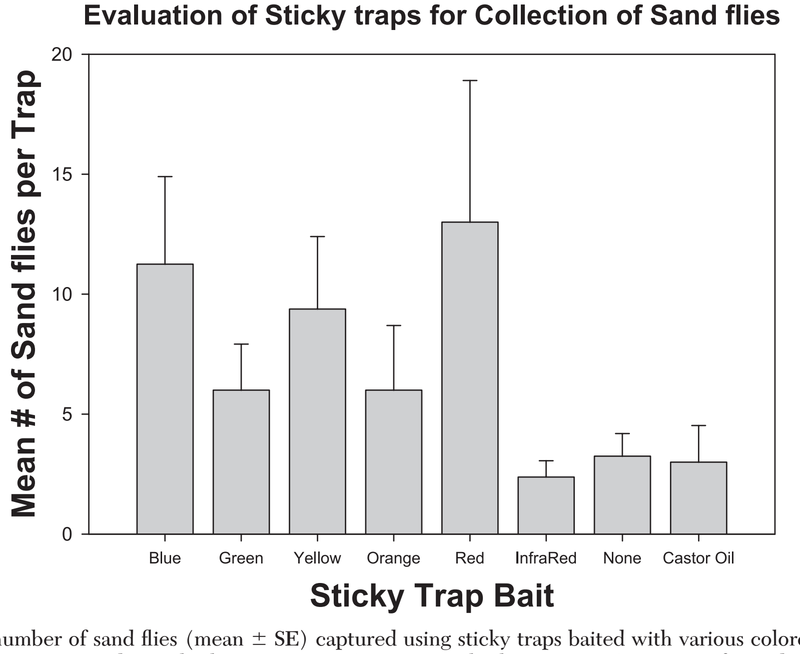

Fig. 2. Total number of sand flies (mean ± SE) captured using sticky traps baited with various colored chemical light sticks. Three-way ANOVA and a multiple comparison (Duncan multiple range test) were performed after log $(x + 1)$ transformation. Means having the same letter are not significantly different ($n = 8$ nights; $\alpha = 0.05$).

the number of sand flies captured on oiled cards with and without light sticks, although the species diversity was greater on the lighted sticky traps. In Kenya, Mutero et al. (1991) found updraft traps were more effective at collecting several species of sand flies near rodent burrows than downdraft traps. Although not statistically significant, the updraft CDC trap evaluated in our study was also more effective at collecting sand flies than the standard downdraft CDC trap.

We found that the UV-CDC trap was the most effective trap evaluated for the collection of large numbers of sand flies. Use of the UV-CDC trap is warranted if the primary objective of a surveillance program is to collect maximum numbers of sand flies to provide the best estimate of field infection rates of a particular pathogen (e.g., *Leishmania* parasites or sand fly fever virus) or to determine whether a particular species of sand fly is present in an area. However, because of the large number of nontarget insects in the UV-CDC trap and the corresponding increase in sample processing time, we feel that the standard CDC style downdraft traps are adequate in the majority of surveillance efforts. Although the updraft CDC trap collected more sand flies than did the downdraft CDC trap, the samples in the updraft trap tended to be in worse condition than those in the downdraft trap (presumably because of repeated contact of dead and moribund specimens with the fan blades at the bottom of the trap). Many other studies have reported that sticky traps baited with chemical light sticks or paper cards coated with oil are effective at collecting sand flies; however, we found that standard CDC light traps were much more effective than the sticky traps (with or without chemical lights) that we evaluated. Future work should focus on evaluating newer trap technologies that incorporate CO_2 or other attractants for the collection of phlebotomine sand flies.

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